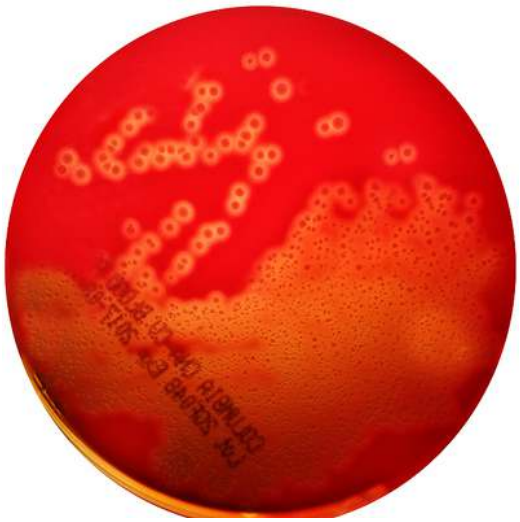
INSTRUCTIONS FOR USE

COLUMBIA CNA-CV BLOOD AGAR

Ready-to-use plates



Streptococcus pyogenes on
Columbia CNA-CV Blood Agar

1 - INTENDED USE

In vitro diagnostic device. Selective medium for the isolation of streptococci and enterococci from clinical specimens containing mixed flora and for determination of bacterial haemolytic model.

2 - COMPOSITION - TYPICAL FORMULA *

Peptocomplex	10 g
Tryptose	10 g
Peptone	3 g
Maize starch	1 g
Sodium chloride	5 g
Agar	12 g
Defibrinated sheep blood	50 mL
Nalidixic acid	15 mg
Colistin	10 mg
Crystal violet	2 mg
Purified water	1000 mL

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Columbia blood agar with 10 mg/L of colistin and 15 mg/L of nalidixic acid was first described in 1966 by Ellner, Stoessel, Drakeford and Vasi[1] of the Columbia University, who combined meat and casein peptones, antibiotics and defibrinated sheep blood into one medium for the isolation of Gram-positive cocci. Addition of crystal violet has been devised for suppressing the growth of staphylococci.[2]
Columbia CNA-CV Blood Agar is a selective medium intended for the isolation and haemolytic properties determination of streptococci and enterococci, particularly when Gram-negative bacteria (e.g. *Pseudomonas*, *Proteus*, *Klebsiella*) and staphylococci are present in the specimens and tend to overgrow on conventional blood agar plates.[3,4]
Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, maize starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth. Colistin, a polypeptide antibiotic of the polymyxin group, and nalidixic acid, a first-generation quinolone, are primarily active against Gram-negative bacteria, crystal violet suppresses the growth of staphylococci. The presence of sheep blood enables the determination of haemolytic pattern, as a useful tool for the orientation of bacterial identification.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Columbia CNA-CV Blood Agar	Ready-to-use plates	541363	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, controlled atmosphere generators and jars, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Columbia CNA-CV Blood Agar plates can be directly inoculated with clinical specimens collected from various normally non-sterile human sites such as ear, upper respiratory tract, genital tract, pus and exudates.[5,6] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[5]

8- TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions with or without 5-10% CO_2 and record the results after 18-24 and 48 hours.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies. Streptococci and enterococci produce growth with β-haemolytic or α-haemolytic or non-haemolytic colonies, that tend to take a bluish colour due to the accumulation of crystal violet. Staphylococci and Gram-negative bacteria are partially or totally inhibited.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 18-24H / A or CO_2	growth, beta haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 18-24H / A or CO_2	growth, alpha haemolysis
S.aureus	ATCC	25923	35-37°C / 18-24H / A or CO_2	inhibited
P.mirabilis	ATCC	12453	35-37°C / 18-24H / A or CO_2	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

11- PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to-use plates of Columbia CNA-CV Blood Agar is tested for productivity, selectivity and haemolytic pattern.
Productivity is tested by semi-quantitative ecometric technique with the following target strains: *S.pyogenes* ATCC 19615*, S. pneumoniae* ATCC 6305*, S.aureus* ATCC 25923 and *P.mirabilis* ATCC 12453. After incubation at 35-37°C for 18-24 hours in aerobic atmosphere the types of haemolysis and the amount of growth is evaluated and recorded. All strains must show a good growth with typical haemolysis. The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target organisms*, P.mirabilis* ATCC 12453 and *S.aureus* ATCC 25923. After incubation at 35-37°C for 18-24 and 48 hours in aerobic atmosphere, the growth of all non-target strains is totally inhibited.

12 - LIMITATIONS OF THE METHOD

- Due to the carbohydrate (starch) content of Columbia CNA-CV Blood Agar, some β-haemolytic streptococci may exhibit an α-haemolytic reaction around a small clear zone of β-haemolysis or may exhibit weak haemolytic reactions.[4]
- The growth and type of haemolysis depends on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic models other than expected.
- The colony diameter is generally smaller than that observed on Columbia Blood Agar Sheep.
- Some Gram-negative bacteria and yeasts could be resistant to the CNA antibiotic mixture and may not be inhibited on this medium.
- Some Gram-positive bacteria other than streptococci and enterococci may also grow, depending on their sensitivity to inhibitors.
- Since some pathogens require carbon dioxide for growing, it is preferable to incubate the plates with 5 -10% CO_2.
- Due to the crystal violet content, the appearance of the medium after incubation is darker than Columbia Blood Agar or Columbia CNA Blood Agar.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic or haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the ready-to use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

1. Ellner PD, Stoessel CJ, Drakeford E, Vasi, F. A new culture medium for medical bacteriology. Am. J. Clin. Path 1966; 45: 502-504.
2. Fry BA. Basic triphenylmethane dyes and the inhibition of glutamine synthesis by Staphylococcus aureus. J Gen Microbiol 1957; 16: 341-349.
3. Atlas D, Snyder J. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.345.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015.
6. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/09
Revision 2	Removal of obsolete classification	2023/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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